Research Design and Methods
This investigation involves two "phases", development of the device prototype and testing in an animal model. These "phases" will be conducted concurrently and not in sequence. The process will be iterative and heuristic. That is, a number of prototypes will be developed until benchtop performance tests demonstrate the ability to fully expand the ends of the stent while the center portion remains undilated or minimally expanded. Once this is satisfied, the stent prototype will be coated with PTFE and its implantation attempted, with a complete angiographic and hemodynamic evaluation. The next prototype will be modified to adapt to any shortcomings with the first one. We have estimated that we need 18 evaluable study animals to detect differences in treatment effect between the two proposed treatment groups, and have included two extra in consideration that the first prototype may not be perfect, as well as for other potential drop out. We estimate that we may develop up to a half dozen stents for benchtop testing.

Proprietary Prototype Development
The prototype stents will be produced by direct ultraviolet (UV) ablation of portions of hollow 316 stainless steel tubing. The first stents we produce will be made using the design parameters we developed in the preliminary studies. Figure 5 is an illustration of the expanded stent elements. The center portion of the stent will be more ridged because the struts will be wider and they will have thicker shoulders. We will test the expansion of these stents by expanding them with a conventional balloon catheter and measuring the expanded diameter of the stent at eleven locations along the length of the stent. This will be done at 25%, 50%, 75%, 100% and 125% of the designed expansion pressures. We will be looking for uniform and symmetric expansion of the stent, indications of any damage to the expansion balloon, problems with balloon deflation, and problems with balloon extraction. These experiments will be done with the catheter in free air and with the catheter in a simulated pig artery. It is expected that these initial studies will provide technical data which will assist in the next iteration of the stent design. As in conventional stent production, post processing operations may be required to address specific problems such as sharp edges on the struts. The ablation method of stent production will have a much smaller effect on the metallurgy of the stent and we do not expect any post processing will be required to remove slag.
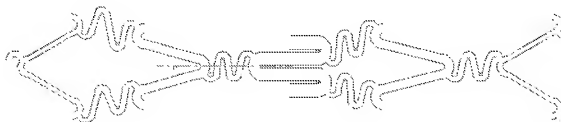

Figure 5. Illustration of expanded stent elements

The controlled ablation metal removal will allow some design freedom not available when using other production methods. One example of this is we can remove a portion of the wall material at the ends of the stent so that the ends will expand with a lower balloon pressure than the middle portion of the stent. Many conventional stents are built with struts having a square crossection (i.e. the width and thickness of the strut are nominally equal). In our first design we will reduce the width of the strut as we go from the middle of the stent to the ends. We expect that information from the testing of those stents will indicate how much thinner the struts will have to be made to meet the design requirements of an expanded diameter of 7mm at the ends and 2mm at the center. It is our goal to design the stent so that the desired lumen diameter on deployment can be achieved with a single balloon design. The test procedure for the

initial design will be updated to assure testing is complete and then the redesigned stents will be produced and tested.

Once the prototype stent framework has been developed, a "skin" will be applied to improve the ability to achieve a pressure gradient across the prosthesis, to reduce turbulence in the lumen and between the lumen and artery wall, and to reduce the likelihood of mural thrombus forming and embolizing downstream into bowel arteries. The "skin" will be expanded polytetrafluoroethylene (ePTFE), which is ideal for this application as it can be bonded securely by a proprietary patented process developed by Atrium Medical (Hudson, NH) while being low in bulk and preserving many of the advantages of a low-profile system. These include small access holes and trackability/deliverability to the artery of interest. The PTFE encapsulation is completed by taking a small ePTFE tube typically 1 mm in diameter and a little longer than twice the length of the stent. This tube is placed within the stent, with half of the excess length of material extending from either edge of the stent. Then the additional length of material is rolled over the outer edge of the stent to cover the outside of the stent. The outer layer is overlapped in this process. Once the PTFE is overlapped it is then sintered where the PTFE material binds together to form one piece. A quote for coating 10 stents with ePTFE is included from Atrium Medical. After coating the stents with PTFE, they will be gas-sterilized. The coated stents will be retested by the above procedures to assure design parameters can be met with the coated stents and to assure the coating remains intact through the stent expansion process.

Prototype Testing: Animal Experiment
The animal experiment will be conducted in an 11,000 square foot animal facility at Rhode Island Hospital (Providence, RI) including an operating room, angiographic and fluoroscopic imaging equipment. The protocol will be submitted to the Rhode Island Hospital Animal Care and Use Committee, and Dr. Murphy will be the principal investigator. A collaboration letter from Luis Sousa, Ph.D., director of the animal laboratory at Rhode Island Hospital, is included in this application. All animal care will be provided per Rhode Island Hospital standard operating procedures and policy which are accordance with the U.S.D.A Animal Welfare Act Regualtions done according to the ILAR *Guide for Care and Use of LaboratoryAnimals*. The animal facility has Full-Accreditation with the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC-International).

Once the reducing stent-graft prosthesis is developed, the experiment in swine will be conducted. Prior to randomization, stent placements will be attempted in adult farm swine. When a suitable stent-graft is developed, the survival experiment may be conducted in smaller animals, such as Yucatan or Hanford minipigs, if placement in their smaller arteries is deemed feasible at the time. Swine are an excellent model of obesity[23] and will gain about a pound a day when fed liberally. They are also large enough to be treated with the prosthesis, in contrast to smaller animal models. Pig mesenteric arterial anatomy is similar to humans and has been well-characterized angiographically and pathologically[5]. In the pig, a celiac, "cranial" mesenteric artery, and "caudal" mesenteric arise from the abdominal aorta, with the "cranial" mesenteric artery supplying most of the small intestine to the ascending colon[6].

Pigs will be observed for dietary intake for 3 days prior to the procedure by weighing all dispensed food and subtracting that not consumed, and pigs will also be weighed pre-procedure. On the day of the procedure, animals will receive a dose of 325 mg aspirin orally, and then anesthesia will be induced with Telazol (tiletamine hydrochloride 50 mg/mL, zolazepam hydrochloride 50 mg/mL) 6 mg/kg and Rompum (23 mg/mL, (xylazine hydrochloride) by intramuscular (IM) injection and sodium pentothal to effect (20mg/kg) intravenously (IV), and then maintained under isoflurane gas anesthesia. Preoperatively, the animals also will be given Atropine 0.05 mg/kg, and buprenorophine (0.01 mg/kg intramuscularly (IM)) for pain. They will be placed supine in a sterile operating room environment, and the skin disinfected first clipping the hair around the surgical area in both groin regions followed with alternating scrubs with

providone-iodine scrub and alcohol, and then with povidone-iodine solution. Access to one or both femoral arteries will be performed by cut down after assurance of adequate anesthesia. Once the common femoral arterie(s) are dissected free, vessel loops will be placed proximally and distally. Puncture of the common femoral artery(ies) will be done using an 18 G or 19G hollow-core needle, and a guide wire placed under fluoroscopic guidance into the upper abdominal aorta. A vascular sheath will be placed and connected to a flush of half normal saline at an infusion rate of 15-30 milliliters/hour. A flush catheter will be placed through the sheath and a flush abdominal aortogram obtained to map out the blood supply to the intestine. Selective catheterization of the gastroduodenal artery and superior ("cranial") mesenteric artery will be done with a shaped catheter and arteriography done of those circulations.

Randomization to sham procedure or stent-graft placement will be done after diagnostic arteriography using an envelope system. For sham treatment group animals, the catheters will then be removed and wounds closed using standard techniques. For active treatment group animals, occlusion of the gastroduodenal artery will be done proximally with coils placed transcatheter. The endovascular prosthesis will be placed in the superior ("cranial") mesenteric artery and deployed. The endoprosthesis is designed to inflate like a dumbbell, narrow in the middle but wide at its ends. The approximate lumen diameter on deployment will be 2 mm in the middle and 6 or 7 at the ends. Pressure measurements will be done distal and proximal to the prosthesis before and after injection of 25 mg of the alpha blocker tolazoline into the SMA. Tolazoline is a vasodilator and will augment any pressure gradient, or bring out a pressure gradient where none existed in the resting state. The endograft lumen diameter will then be increased using angioplasty balloons if needed so that there is no or minimal gradient at rest (less than 10 mm Hg mean). Final pressure gradients at rest and after tolazoline will be recorded. Subsequently, wounds will be closed using absorbable sutures beneath the skin (2-0 through 4-0 Vicryl, Ethicon/Johnson&Johnson, Warren, NJ) and staples for the skin, and animals will then emerge from anesthesia.

The animals will be monitored for two hours post-op to monitor behavior and level of activity to make sure that the animals have completely recovered from anesthesia. They will be evaluated q 12 hours and given analgesia, buprenorphine 0.01mg/kg IM for 24 hrs for pain. They will receive aspirin 325 mg orally each day for platelet inhibition, anticoagulation is not done in clinical practice for humans with stents grafts and won't be done. The pigs will be closely monitored for 7 days for changes in activity, behavior, eating and watering habits. If the pig is found to not be eating or to be experiencing any discomfort, the veterinarian will be consulted and additional doses of Buprenorphine may be administered at their discretion. Euthanasia will be performed in an instance were the animal is found to be in significant distress or discomfort, continuing decreased food/water intake, or exibiting lethargy despite treatment at the discretion of the veterinarian in consultation with the investigators.

Thereafter, the animals will be checked daily for any changes in activity, behavior, eating habits, urine or feces output. If any change is noted, appropriate evaluation will be done to determine if the animal is experiencing any pain or if there is an infection present (infection will be evaluated by visual examination of surgical sites for abnormal changes as well as temperature evaluation). They will be monitored for dietary caloric intake for 3 day periods at one week and one month post-procedure. Weighers will be trained on the laboratory standard method of weighing, including weighing on a consistent time of day, and will be blinded to the treatment group. After 60 days, pigs will be weighed and sacrificed. Gross inspection of the bowel and mesenteric artery endoprosthesis done for descriptive purposes, including the presence of bowel ischemia, stricture, infarction, intraarterial thrombus or intimal hyperplasia.

Statistical Analysis:

Data will be collected in laboratory notebooks and entered into a computer database (Access 2000, Microsoft, Redmond, WA) designed for this project. Data will be analyzed using StatView v.5.0.1 statistical software (SAS Institute, Cary, NC).

Primary endpoint:
Change in weight will be compared between treatment groups using repeated measures ANOVA with Group as a fixed effect. Sample size was estimated based on the anticipated difference between groups at 60 days (t-test). It is anticipated that the use of repeated measures ANOVA at analysis will be more sensitive and so the calculated sample size represents a conservative estimate of that required. The following assumptions are made:

- The mean weight for the population of pigs at baseline is 160 lbs
- After one month of ad lib feeding, the control group mean weight will be 170 lbs
- After one month of ad lib feeding, the treatment group mean weight will be 150 lbs
- The group standard deviation is 12 lbs
- The effect size, or standardized difference, $=\{(170\text{-}150)/12\}=1.67$

$H_o: \mu_{SH} = \mu_{ST}$
$H_a: \mu_{SH} \neq \mu_{ST}$

Where μ_{ST} is the primary endpoint estimate for the reducing stent-graft group and μ_{SH} is the primary endpoint estimate for the sham group at 60 days. Rejection of the null hypothesis will signify that the weight change between the two groups is significantly different. Given the above, with an α of .05 and power of 90%, a sample size of 18 (9 per group) evaluable subjects is needed (EaST 2000 software, Cytel Corporation, Cambridge, MA). We have inflated the sample size by two to account for study subject drop out such as for example due to technical failure of stent-graft implantation procedure and complications of either treatment, or drop out due to other unanticipated causes.

Secondary endpoints:
1. Estimate of dietary caloric intake—Pigs will be fed *ad lib*, and excess amounts of feed will be left in the pen with each pig. Caloric intake will be calculated by measuring standard feed prior to filling the feed dispensing bin, and then at the end of a three day period measuring how much is left in the bin and in the pen, and therefore how much was consumed. Caloric equivalents will be calculated according to the feed manufacturer's information. The change in average caloric intake over the 3 day period pre-intervention compared with one week and one month later will be compared using a t-test.
2. Adverse events—Adverse events like death, bowel infarction, diarrhea, aversion to food, infection, and reduced activity (water intake, bladder and bowel function) will be recorded and reported so that risks of the proposed can be understood, and any benefits in terms of weight loss be understood in the context of the challenges posed by these events

Key Personnel
Dr. Timothy Murphy is the Director of Quequechan Engineering, Inc., and an interventional radiologists practicing in Providence, Rhode Island. He is the Director of the Vascular Disease Research Center at Rhode Island Hospital, and a Professor, Research Track, of Diagnostic Imaging at Brown Medical School. He has over 13 years of experience as an interventional radiologist and performs procedures similar to those proposed as part of this experiment on a regular basis. He is a fellow of the Society of Interventional Radiology, the American Heart Association, the Society of Vascular Medicine and Biology, and the American College of Radiology. He is the principal investigator of the CLEVER multicenter

randomized clinical trial (Claudication: Exercise Vs. Endoluminal Revascularization, NHLBI R01 HL077221), and co-principal investigator of the CORAL Study (Cardiovascular Outcomes with Renal Atherosclerotic Lesions, NHLBI R01 HL071556-01). In addition to participating in prototype design and development, Dr. Murphy will perform the animal experiment as principal investigator, will collect and analyze data, and report study results.

Dr. Lamar Bullock received a Ph.D. in physics from Michigan State University and is an Adjunct Professor of Physics at University of Massachusetts-Dartmouth, and is the director of the photonics laboratory that includes the IX-300 Micro-Machining Laser. Dr. Bullock was formerly president of Boston Laser Technology, where he developed a unique excimer laser-based manufacturing process. He also designed and built laser imaging equipment to apply this process to volume manufacturing of a coronary stent for a company, Buckbee-Mears (St. Paul, MN), that was developing stents for Cordis/Johnson&Johnson (Warren, NJ), one of the largest manufacturers of vascular stents in the world. This project required close coordination with the stent designers to make optimal use of a new laser-based manufacturing process.

Cong Wang is a graduate student in Physics at the University of Massachusetts-Dartmouth. He received his bachelor's degree with a major in Applied Chemistry, Beijing University of Technology in 2004, and has done research in the Institute of Physics, Chinese Academy of Science, on non-materials and superconductivity. As an intern at the University of Massachusetts Advanced Technology and Manufacturing Center, Cong Wang has acquired skills in the operation of the Excimer Laser Micro-Machining Center and CAD design of stents.